Simultaneous Onset of Type 1 Diabetes Mellitus in Identical Infant Twins with Enterovirus Infection

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This report describes classical Type 1 insulin deficient diabetes mellitus (DM) arising in twins aged 14 months, both of whom had evidence of enterovirus infection. The diagnosis of Type 1 DM was made in the second twin within 12 days of the first. Enterovirus infection was detected in each twin at diagnosis by polymerase chain reaction (PCR). Both twins were negative for enterovirus by PCR 5 months following diagnosis, although both were then positive for islet cell antibodies. Sequencing of the amplicons produced by PCR suggested that the viruses from each twin were not the same but that they were both variants related to echovirus 6. © **1998 John Wiley & Sons, Ltd.**

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Introduction

There has recently been a renewal of interest in the association between enterovirus infection and the onset of Type 1 DM[1–3] but there have been few relevant studies relating to children under 5 years of age. Gamble and Cumming[4] first showed that coxsackie IgM antibodies were detected more frequently in newly diagnosed diabetic children under the age of 2 than in control children. Similar findings were also reported for children under the age of 3 in a Finnish childhood diabetes survey, using antibody methods to detect evidence of virus infection.[5] Finally, Clements and his coworkers, using PCR analysis, showed that up to 50 % of recently diagnosed diabetic children under the age of 6 were positive for enteroviruses of the coxsackie type at onset.[6] There have also been occasional reports of Type 1 DM arising over short periods of time among members of a single family, suggestive of an infective precipitant, for example the simultaneous onset of Type 1 DM in teenage siblings who had both acquired coxsackie B infections.[7] There is also an account of diabetes arising among different members of the same family over a period of several months, again accompanied by coxsackie B4 infection.[8] Here, we report for the first time the simultaneous onset of diabetes in twins aged 14 months associated with an echovirus 6 infection.

Clinical History

White female twins were born following an uneventful pregnancy at 36 weeks gestation by elective Caesarian section. Their birth weights were 2.67 kg (twin 1) and 2.04 kg (twin 2). The twins remained healthy for 14 months and then twin 1 was admitted to hospital with a 6-day history of diarrhoea, intermittent fever, polydipsia and weight loss. She was suffering from diabetic keto-acidosis. Blood glucose was 31 mmol l^{-1}, pH 7.24, standard bicarbonate concentration 6.8 mmol l^{-1}. After treatment with intravenous fluids and a continuous infusion of soluble insulin, she made an uneventful recovery and was discharged home on twice daily biphasic insulin. Twelve days later, twin 2 was admitted with a 1-day history of polyuria and irritability. Her blood glucose was 25.2 mmol l^{-1} on admission. Diarrhoea was absent. Now 14 months later, the twins are well controlled on about 0.8 U kg^{-1} of biphasic insulin daily.

Methods

Methods used to detect and identify viral RNA by PCR using primers from the enteroviral 5′ non-translated region have been described in detail elsewhere.[6,9] These were applied to serum samples. Serum samples were available from each twin at diagnosis and 5 months later. Maternal blood was also taken at the 5-month

Abbreviations: GAD glutamic acid decarboxylase, JDF-u Juvenile Diabetes Foundation units, PCR polymerase chain reaction

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point. Serum from 10 non-diabetic children living in the Greater Manchester area and randomly selected from children attending Booth Hall Hospital were also available. Serum samples were kept frozen at –20 °C until used. Under the conditions used, the signal appears to be stable and the nested PCR picks up 10 copies of the sequence or less in the 200 μl volume that is processed. Conventional islet cell antibodies (ICA) were measured by indirect immunofluorescence (IFL), on cryostat sections of blood group O pancreas as described.[10] Positive samples detected with undiluted serum were titrated to end point in doubling dilutions. Local standard sera calibrated to 2, 4, 6, 8, 16, 32, and 80 were included in each assay. End point titres of test samples were converted to JDF units (JDF-u) by comparison with a standard curve of log JDF-u versus log end-point of the standard sera.[11] The threshold of ICA detection was 5 JDF-u. GAD antibodies were assayed by an *in vivo* labelled human recombinant GAD-65 using a recombinant cDNA cloned GAD-65 (kindly provided by Professor A. Lernmark to provide a pseudo RNA first).[12] Positive values for GAD antibodies were designated as above 10 LH-u. IA-2ic antibodies were measured as for GAD antibodies as recently described.[13] A recombinant cDNA clone IA-2ic was kindly provided by Dr Michael Christie. Positive values for IA-2ic antibodies were above 10 LH-u.

Antibodies to thyroglobulin (Tg) and thyroid microsomes (Tm) were detected by indirect haemagglutination using a commercial kit (Serodia, Japan). The threshold titre for antibody positivity was 1/20 for Tg-Ab and 1/400 for Tm-Ab. Antibodies to gastric parietal cells (GPAb) and to cortical adrenal gland were detected with undiluted serum by indirect IFL using 4 μm sections of human blood group O stomach and adrenal respectively.[14] Non-organ specific antibodies (i.e. antinuclear, antimitochondrial, anti-smooth muscle, etc), were measured in 4 μm cryostat sections of rat kidney and liver with sera diluted 1:10 as previously described.[15]

Results

No specimens were available for routine virus isolation so that investigations were limited to carrying out PCR testing on serum samples only. In addition there was insufficient serum from the twins to allow for routine virus antibody testing at the time of diagnosis. Sequences from the enterovirus 5′ non-translated region were detected by PCR in serum taken at the time of diagnosis of the diabetes but were not present in serum 5 months later. Serum from the mother was negative for enteroviruses at the time of second sampling, the only maternal sample available. Ten serum samples from children under the age of 2 and taken at the time of the twins' diagnosis were all negative for enteroviruses. Five months after diagnosis, samples of serum from twin 1 were positive for ICA (30 JDF-u), GAD antibodies (2321 LH-u), and IA-2ic antibodies (62 LH-u). For twin 2, ICA were positive (45 JDF-u), as were GAD antibodies (132 LH-u) and IA-2ic antibodies (22 LH-u). However, serum samples from both twins were negative for thyroid, gastric parietal cell, adrenal and non-organ specific antibodies.

The twins were identical. Their HLA status was A24,B7,B39. Their DQB1 status was ∗0302,0603. That of their father was DQB1,∗0302,0604 and the mother was ∗0503,0603. Sequences of the non-translated region of the amplicons derived from each twin were compared with sequences from a variety of enteroviruses and found to be most closely related to echovirus 6 (Figure 1). Twin 1 has 6.7 % and twin 2 4.5 % divergence from the published sequence of echovirus 6. There were no common features between the sequences found in each twin to suggest a single immediate source of infection.

Discussion

Although Type 1 DM has often been diagnosed among the siblings of children who are already diabetic, the time interval between the first and second diagnosis usually ranges from months to years. In this instance, two twins appear to have acquired diabetes simultaneously at a very early age. It may be relevant that both twins are homozygous for HLA A24 which is said to be associated with the rapid progression of Type 1 diabetes. Moreover both twins showed evidence of echovirus 6 infection at the time of diagnosis and had no evidence of infection 5 months later.

There have now been many reports linking enterovirus infection with the onset of Type 1 DM, particularly in very young children. Such infections may begin *in utero*, as has been suggested in two recent Scandinavian studies.[5,16] In this instance we were unable to obtain blood specimens from the mother of the twins taken during pregnancy to ascertain whether or not there was an enterovirus infection which might have persisted.

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                201                                                 250
Echovirus 6     TCAATAAGCT GCTCACGCGG CTGAAGGAGA AAGCGTTCGT TACCCGGCTA
     twin 1     n..........  ...nG.....  ..........  ..AA.....  ........C.
     twin 2     ......G...  ..........  ..........  ..........  ..T.......

                251                                                 300
Echovirus 6     GTTACTTCGA GAAACCCAGT ACCACCATGA AGGTTGCGCA GCGTTTCGCT
     twin 1     A..........  ..........  ...G......  .T......AG .T........
     twin 2     ..........  ......T...  .A........  ..........  ..........

                301                                                 350
Echovirus 6     CCGCA.AACC CCAGTGTAGA TCAGGTCGAT GAGTCACCGC GTTCCCCACG
     twin 1     .A...C....  ..........  ..........  ..........  ..........
     twin 2     ..........  ..........  .....C....  ...C......  .....T....

                351                                                 400
Echovirus 6     GGCGACCGTG GCGGTGGCTG CGTTGGCGGC CTGCCTATGGG GCAACCCAT
     twin 1     ..T.......  ..........  ..........  .....C.....  ..........
     twin 2     ..........  ..........  ..........  .........A.  .A...T...

                401
Echovirus 6     GGGACGCTTC AATACTGACA TG
     twin 1     .......C..  ..........  ..
     twin 2     ..........  ..........  ..
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Figure 1. Alignment of the 5′ non-translated region of echovirus type 6 from the GenBank database with sequence derived from PCR amplicons of each twin. The comparison was performed using the pileup program included in the Genetics Computer Group Package at the University of Glasgow

In most instances, enteroviruses associated with the onset of diabetes appear to be members of the coxsackie B group; all six members of this group have been implicated.[1,2] However, in our twins the viruses involved are more closely related to echovirus 6. Despite sporadic reports of echovirus 6 infections in the UK, there was no evidence for a major outbreak of echovirus 6 infection in the Manchester area at the time of the twins' diagnosis in the PHLS returns. Earlier work has suggested that echoviruses like coxsackie viruses can be diabetogenic in some patients[17] and in animals.[18] Sequence data was derived from the amplified 5′ non-translated region in our study. Although the viruses in the twins were clearly related to each other and to echovirus 6, the data do not make it possible to determine the relationship between the time of onset of the diabetes and of the initial virus infection. The children could have been infected from a common source, perhaps some time before the diagnosis of diabetes after which alterations in the virus nucleic acid sequence could have occurred, explaining the differences between the two. Such an infection with echovirus some time before presentation could conceivably be a trigger for islet damage.

Alternatively, the children could have been infected from independent sources or twin 1 might have infected twin 2. It is also not possible to exclude a persistent infection. However the fact that both twins were PCR negative after 5 months diagnosis makes long-term persistence unlikely. So far only a small region of the sequence has been examined. It remains to be seen whether sequences associated with diabetogenicity might be present in both viruses in parts of the genome not so far investigated.

The variety of islet cell antibodies detected in the twins were all positive 5 months after the onset of diabetes, when there was no evidence of enteroviral infection. Whether there is a relationship between a possible enterovirus infection of the pancreatic islet cells and the break of immunological tolerance to corresponding target autoantigens must remain speculative.

In conclusion, although the data on these twins are limited, the work provides another interesting example of the association between enterovirus infection and the onset of diabetes at a very early age. Since, as already indicated, echoviruses are capable of damaging the islets of Langerhans, it is a reasonable assumption that they were aetiologically important in this instance.

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